Data Evaluation Report on the Acute Toxicity of BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil) to Fish (*Onchorynchus mykiss*)

PMRA Submission Number: 2008-0432
PMRA Document ID: 1662895 EPA MRID Number: 47560401

Data Requirement:		
	PMRA Data Code	9.5.4
	EPA DP Barcode	349851
	OECD Data Point	IIIA 10.2.2.1
	EPA MRID	47560401
	EPA Guideline	OPPTS 850.1075

Test material: BAS 781 02 H **Purity:** 54.6% (BAS 656 H; Dimethenamid-P) and 6.2% (BAS 800H)
Common name: Dimethenamid-P formulation
Chemical name: IUPAC: BAS 656 H: (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide; not reported for a.i. Saflufenacil
CAS name: Not Reported
CAS No.: BAS 656 H: 163515-14-8; not reported for a.i. Saflufenacil
Synonyms: None Provided

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 11/11/08

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 11/17/08

Primary Reviewer: Anita Pease **Date:** 06/09/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Minderhout, T., T.Z. Kendall, H.O. Krueger and C. Holmes. 2008. BAS 781 02 H: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory report number 147A-239. Study sponsored by BASF Corporation, Research Triangle Park, NC. Study completed August 26, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to BAS 781 02 H (formulation containing 54.6% Dimethenamid-p and 6.2% Saflufenacil) at nominal concentrations of 0 (negative control) 0.63, 1.3, 2.5, 5, 10, 20 and 40 mg/L under static conditions. The 96-h LC_{50} was 17.7 mg form/L. The EC_{50} and NOAEC values, based on mortality and sub-lethal effects, were 7.1 and 2.5 mg form/L, respectively. Based on the LC_{50} of the formulated product, BAS 781 02 H would be classified as slightly toxic to rainbow trout on an acute toxicity basis, in accordance with the classification system of the U.S. EPA.

It can be concluded that dimethenamid-p, not saflufenacil, contributes to the toxicity of the BAS 781 02 H formulation, based on comparison of results to rainbow trout 96h LC_{50} for technical dimethenamid-p of 6.3 mg a.i./L (e-Pesticide manual; MRID 44332227) and technical saflufenacil of >112 mg a.i./L (IIA 8.2.1.1; MRID: 47127904; PMRA: 1547213).

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): mean wet weight- 0.77 (0.51-1.04) g; mean body length- 4.3 (3.6-4.6) cm
Test Type (Flow-through, Static, Static Renewal): Static

Based on the BAS 781 02 H formulation concentrations; uncorrected for active ingredient purity

LC_{50}: 17.7 mg form/L	95% C.I.: 10.0-40.0 mg form/L
EC_{50}: 7.1 mg form/L	95% C.I.: 5.0-10.0 mg form/L
NOAEC: 2.5 mg form/L	
Probit Slope: N/A	95% C.I.: N/A

Endpoint(s) Affected: Mortality and sub-lethal effects

Data Evaluation Report on the Acute Toxicity of BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil) to Fish (*Onchorynchus mykiss*)

PMRA Submission Number: 2008-0432
PMRA Document ID: 1662895 EPA MRID Number: 47560401

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD Guideline for Testing of Chemicals, 203: *Fish, Acute Toxicity Test*; U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines, OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine*; and ASTM Standard E729-96: *Standard Guide for Conducting Acute Toxicity Tests of Test Materials wish Fishes, Macroinvertebrates, and Amphibians*. The following deviations from OPPTS 850.1075 were noted:

1. The TOC and particulate matter concentration of the dilution water were not specified.
2. The total length of the ten negative control fish measured at test termination ranged from 3.6 to 4.6 cm indicating that some of the fish used in the study were slightly shorter than the recommended 5.0 $\pm$ 1.0 cm total length. Although two of these fish were shorter than the recommended total length stated in the protocol, the mean total length and mean wet weight of fish met the recommended total length (5.0 $\pm$ 1.0 cm) and the required wet weight (0.1 to 3.0 g) stated in the protocol. They also appeared healthy during the holding period and the length of the longest fish was no more than twice that of the shortest fish.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the GLP Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau 1, October 1999), with the following exception: periodic analyses of well water for potential contaminants were not performed according to Good Laboratory Practice Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil)

Description: Liquid

Lot No./Batch No. : 1632-78 (Batch Number)

Purity: 54.6% BAS 656 H and 6.2% BAS 800 H

Stability of compound under test conditions: Analytical verification of the primary active ingredient, Dimethenamid-P was conducted at 0, 48 and 96 hours. Analysis at test initiation yielded recoveries of 97.9-101.3% of nominal. Recoveries at 48 and 96 hours were all ≥97.6% of initial measured concentrations.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored under ambient conditions.

Physicochemical properties of BAS 781 02 H.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Juveniles

Weight at study initiation: mean wet weight- 0.77 (0.51-1.04) g (control fish at test termination)
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: mean body length- 4.3 (3.6-4.6) cm (control fish at test termination)
EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout

Source: Test organisms were obtained from Thomas Fish Company, Anderson, CA.
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 96-hour range-finding study was conducted with nominal concentrations of 0 (negative control), 0.81, 2.7, 9.0, 30 and 100 mg form/L. Five fish were exposed to each treatment level and mortality after 96 hours of exposure was 0% in the control, 0.81, and 2.7 mg form/L treatment groups, 20% in the 9.0 mg form/L treatment group. and 100% in the 30 and 100 mg form/L treatment groups.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	14 days	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Conditions: (same as test or not)	Same as test	
Feeding:	Fish were fed a commercial fish food (Zeigler Brothers, Inc., Gardners, PA) daily; feeding was terminated 48 hours prior to test initiation.	
Health: (any mortality observed)	No mortalities or diseases were observed during the acclimation period.	
Duration of the test	96 hours	*The recommended test duration is 96 hours.*
Test condition		
Static/flow-through	Static	
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	Test vessels were not aerated	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Stainless steel 38 L 30 L	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm. Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Dilution water was obtained from an on-site well approximately 40 meters deep and was characterized as moderately-hard. Water was passed through a sand filter to remove particles greater than ~25 µm and pumped into a 37,800 L storage tank where the water was aerated with spray nozzles. Prior to use, the water was filtered to 0.45 µm to remove fine particles.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature Intervals of water quality measurement	 138 mg/L as $CaCO_3$ 8.0-8.5 8.0-9.8 mg/L (≥74% saturation) Not Reported Not Reported See Reviewer's Comments None detected 4.2 mg/L as chloride 11.1-13.0°C Temperature, DO and pH were measured daily in all test vessels. Temperature was also continuously measured in a centrally located negative control test chamber.	Conductivity was 355 µS/cm and alkalinity was 178 mg/L as $CaCO_3$ (at test initiation) *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ≥60% during first 48 hrs and ≥ 40% during second 48 hrs; flow-through: ≥60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 °C for coldwater species, 17 or 22 °C for warmwater species, and 22 ± 1 °C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 2 N/A 2	*Recommended number of replicates includes a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	*Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.26 g/L	*Recommended static conditions are ≤ 0.8 g/L at ≤ 17°C and ≤ 0.5 g/L at > 17°C. Recommended flow-through conditions are ≤ 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: Nominal:	0 (negative control), 0.63, 1.3, 2.5, 5.0, 10, 20 and 40 mg form/L	Only concentrations of dimethenamid-p were analytically verified. Given the stability of saflufenacil and dimethenamid-p over the duration of the test and lack of analytical verification for the formulation, all endpoints are reported in terms of nominal concentration.
Solvent (type, percentage, if used)	N/A; a solvent was not used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D with a 30 minute transition period of low light intensity; mean of 331 lux at test initiation	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the study.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization Level of detection	0, 48 and 96 hours 0.183 mg/L 0.00899 mg/L	The active ingredient measured for analytical determination of test concentrations was the primary active ingredient, Dimethenamid-P. The LOQ and LOD were corrected for the percent composition of this active ingredient in the formulation.
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None reported	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Sub-lethal effects	
Observation intervals	4, 24, 48, 72 and 96 hours	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	At test initiation, the 0.63 to 10 mg/L and control solutions appeared clear and colorless. A white foam was observed around the sides of the 20 mg/L test chambers. A white foam was observed throughout the water surface of the 40 mg/L test solutions. At test termination, all test and control solutions appeared clear and colorless in the test chambers.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

One mortality was observed in the nominal 40.0 mg form/L treatment level within 4 hours of test initiation; complete mortality was observed at this level by 24 hours. After 48 hours, 30% mortality was observed in the nominal 20.0 mg form/L treatment level, mortality increased to 35% at 72 hours and finally 65% by test termination. No mortality was observed in the control or nominal 0.63 to 10.0 mg form/L treatment groups. Based on mean-measured concentrations of BAS 781 02 H, the study authors reported no mortality and LC_{50} values of 9.7 and 18 mg form/L, respectively. Based on nominal concentrations, no mortality and LC_{50} values are 10 and 17.7 mg form/L, respectively.

Table 3: Effect of BAS 781 02 H Technical on Mortality of (*Onchorynchus mykiss*).

Nominal Concentrations (mg form/L)	No. of Fish at Start of Study	Observation Period					
		24 Hours		48 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	20	0	0	0	0	0	0
0.63	20	0	0	0	0	0	0
1.3	20	0	0	0	0	0	0
2.5	20	0	0	0	0	0	0
5.0	20	0	0	0	0	0	0
10	20	0	0	0	0	0	0
20	20	0	0	6	30	13	65
40	20	20	100	20	100	20	100
NOAEC	9.7 mg/L* (10 mg form/L)**						
LC_{50}	18 (9.7-40) mg/L* (17.7 (10-40) mg form/L)**						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A- Not applicable
*reported by the study authors (based on mean-measured concentrations)
** calculated by reviewer

B. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the control or nominal 0.63-2.5 mg form/L treatment levels. At 48 hours, all fish in the nominal 5.0 mg form/L treatment level were hyperventilating; however, all fish had recovered by 72 hour and remained normal and healthy throughout the remainder of the test. Within 4 hours of test initiation, two fish in the nominal 10.0 mg form/L treatment level were observed surfacing; no effects were observed at this level at 24 hours, but all surviving fish were exhibiting sub-lethal effects (surfacing and hyperventilating) at 96 hours. At the nominal 20.0 mg form/L treatment level, sub-lethal effects (surfacing, lethargy, loss of equilibrium and lying on the bottom of the test chamber) were observed during all observation periods within the study . The NOAEC and LOAEC values were reported to be 2.5 and 5.0 mg form/L, respectively.

Table 4: Sub-lethal Effect of BAS 781 02 H on (*Onchorynchus mykiss*).

Nominal Concentrations (mg form/L)	Observation Period		
	Endpoints at 24 Hours	Endpoints at 48 Hours	Endpoints at 96 Hours
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
0.63	A.N.	A.N.	A.N.
1.3	A.N.	A.N.	A.N.
2.5	A.N.	A.N.	A.N.
5.0	A.N.	100%- Hyperventilating	A.N.
10	A.N.	35%- Hyperventilating 65%- Surfacing and hyperventilating	55%- Hyperventilating 45%- Surfacing and hyperventilating
20	85%- Loss of equilibrium 10%- On bottom	50%- Loss of equilibrium 50%- Lying on bottom	29%- Lying on bottom 29%- Loss of equilibrium 43%- Hyperventilating
40	Complete Mortality	Complete Mortality	Complete Mortality
NOAEC	2.5 mg form/L		
LOAEC	5.0 mg form/L		
EC_{50}	7.1 (5.0-10.0) mg form/L*		
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A

A.N.- All fish appear normal and healthy
N/A- Not applicable
*calculated by the reviewer

C. REPORTED STATISTICS:

The LC_{50} value and associated 95% confidence interval were estimated using the binomial probability method. The NOAEC value was determined by visual observation of the mortality and observation data. All results were reported based on the mean-measured concentrations of the formulation.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 96-hour LC_{50} value and confidence interval were determined using the binomial probability method via Toxanal statistical software and the no mortality value was verified using Fisher's Exact Test via Toxstat statistical software. The NOAEC was visually determined, based on the appearance of sublethal effects at 48 hours. The EC_{50} value was calculated similar to the LC_{50} value, except comparisons were made based on the number of affected fish relative to the number of surviving fish at the respective level. All analyses were performed using the

nominal test concentrations and expressed in terms of the formulation.

Based on the BAS 781 02 H formulation concentrations; uncorrected for active ingredient purity

LC_{50}: 17.7 mg form/L	95% C.I.: 10.0-40.0 mg form/L
EC_{50}: 7.1 mg form/L	95% C.I.: 5.0-10.0 mg form/L
NOAEC: 2.5 mg form/L	
Probit Slope: N/A	95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were nearly identical to those of the study authors. Additionally, the reviewer calculated an EC_{50} value based on sublethal effects. The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Concentrations of Saflufenacil (BAS 800H) were not measured in this study. The analytical determination was conducted for the primary active ingredient, Dimethenamid-P, only. Analytically verified concentrations of dimethenamid-p were 94-101% of nominal after 96 hours. Although the stability of saflufenacil was not measured under test conditions, it is expected to be stable given measured concentrations were 108-114% of nominal after 96 hours in the rainbow trout study conducted with saflufenacil (IIA.8.2.1.1; MRID: 47127904; PMRA: 1547213). Considering dimethenamid-p was stable, saflufenacil is expected to be stable, and concentrations of the whole formulation were not measured, all biological endpoints are reported in terms of nominal concentrations.

It can be concluded that dimethenamid-p, not saflufenacil, contributes to the toxicity of the BAS 781 02 H formulation, based on comparison of results to rainbow trout 96h LC50 for technical dimethenamid-p of 6.3 mg/L (e-Pesticide manual; MRID 44332227) and technical saflufenacil of >112 mg a.i./L (IIA 8.2.1.1; MRID: 47127904; PMRA: 1547213).

The periodic screening analysis of the dilution water indicated the presence of the following components: calcium (38.7 mg/L), chloride (4.2 mg/L), fluoride (0.55 mg/L), magnesium (14.6 mg/L), potassium (6.97 mg/L), sodium (19.8 mg/L) and sulfate (6.0 mg/L).

The physiochemical properties of the test material were not reported.

The in-phase portion of the definitive toxicity test was conducted from July 17 to July 21, 2008.

G. CONCLUSIONS:

The study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA as it fulfills the requirements for an acute freshwater fish toxicity study. The NOAEC and LC_{50} values were 2.5 and 17.7 mg form/L, respectively. Based on these results, the formulation would be classified as slightly toxic to rainbow trout on an acute toxicity basis, in accordance with the US EPA toxicity categorization.

Based on the BAS 781 02 H formulation concentrations; uncorrected for active ingredient purity

LC_{50}: 17.7 mg form/L	95% C.I.: 10.0-40.0 mg form/L

EC_{50}: 7.1 mg form/L	95% C.I.: 5.0-10.0 mg form/L
NOAEC: 2.5 mg form/L	
Probit Slope: N/A	95% C.I.: N/A

III. REFERENCES:

ASTM, 1980. Standard Practice for Conducting Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. American Society for Testing and Materials, Philadelphia, PA. 25 pp.

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USEPA, 1982. Pesticide Assessment Guidelines, Subdivision E- Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024. Office of Pesticide Programs, Washington, DC. 86 pp.

USEPA, 1985. Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Estuarine Fish 96-Hour Acute Toxicity Test). EPA-540/9-85-009. Office of Pesticide Programs, Washington, DC.

USEPA, 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 54, No. 158: 34067-34074.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

MORTALITY

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
40.0	20	20	100	9.536742E-05
20.0	20	13	65	13.1588
10.0	20	0	0	9.536742E-05
5	20	0	0	9.536742E-05
2.5	20	0	0	9.536742E-05
1.3	20	0	0	9.536742E-05
.63	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10.0 AND 40.0 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 17.69323

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.63	20	0	
2	1.3	20	0	
3	2.5	20	0	
4	5.0	20	0	
5	**10.0**	**20**	**0**	
6	20.0	20	13	*
7	40.0	20	20	*

SUB-LETHAL EFFECTS

CONC.	NUMBER SURVIVING	NUMBER AFFECTED	PERCENT AFFECTED	BINOMIAL PROB. (PERCENT)
20.0.	7	7	100	.78125
10.0	20	20	100	9.536742E-05
5	20	0	0	9.536742E-05
2.5	20	0	0	9.536742E-05
1.3	20	0	0	9.536742E-05
.63	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 5 AND 10.0 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.071068

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.